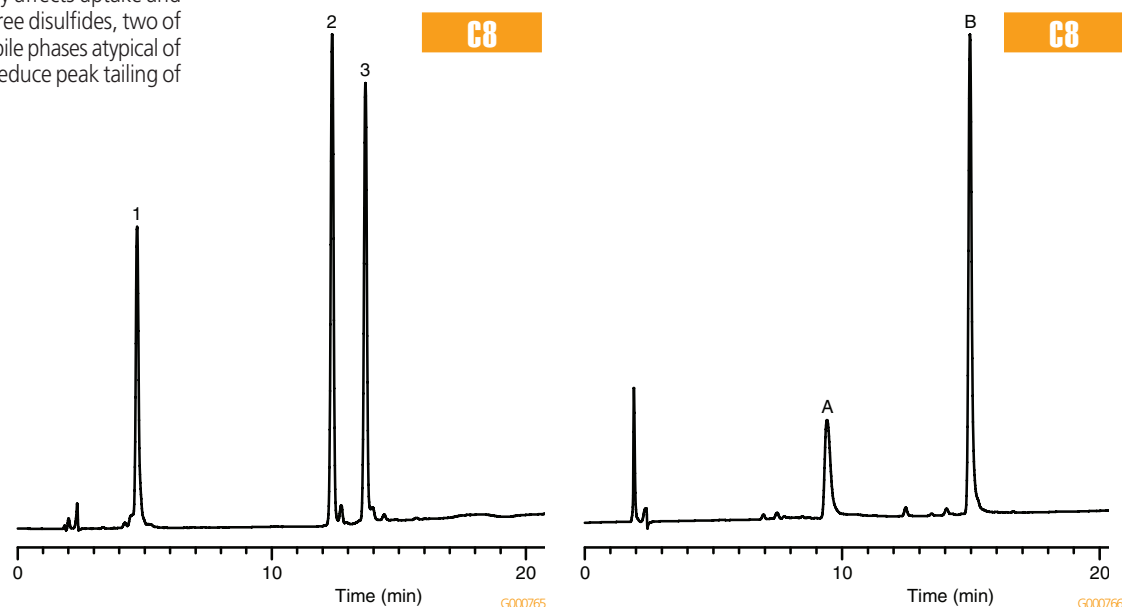


Proteins (Bovine Insulin)

Insulin is a heterodimeric protein hormone that directly affects uptake and metabolism of glucose. The native protein contains three disulfides, two of which serve to crosslink the two subunits. Buffered mobile phases atypical of peptide chromatography are required to dramatically reduce peak tailing of subunit A.



Proteins (Bovine Insulin)

15cm x 4.6mm columns,
5µm particles,
(A) 50mM H₂NaPO₄, 50mM Na₂SO₄, pH 3.0 (w/H₃PO₄): MeCN (95:5)
(B) 50mM H₂NaPO₄, 50mM Na₂SO₄, pH 3.0 (w/H₃PO₄): MeCN (50:50)
33.3% to 73.3% B (20% to 38% MeCN) in 18 min
1mL/min, 35°C, UV, 220nm

- 1 Subunit A, oxidized (sulfonic acids; Sigma® 11633)
- 2 Holoenzyme (Sigma 15500)
- 3 Subunit B, oxidized (sulfonic acids; Sigma 16383)

Holoenzyme (Sigma 15500) reduced and carboxy-methylated

- A Subunit A
- B Subunit B

Tannic Acid from Water or Methanol

Tannic acid (gallotannic acid) is a naturally occurring substance found in tree barks, fruits, and other plant parts. Tannins are derivatives of gallic acid or flavanols, and comprise a broad group of plant-derived phenolic compounds which have the ability to precipitate proteins. Some tannins may be more toxic than others, depending upon the source. Tannins derived from nutgalls are believed to be carcinogens, while those found in tea and coffee are considered non-toxic. These compounds are soluble in water and alcohols and can be extracted from plant matter. Tannins may interfere with the analysis of bioactives in natural product research. Discovery DPA-6S polyamide SPE products can be used to remove tannins from aqueous and methanolic solutions in these applications. In this experiment, 300mg or 600mg of bulk DPA-6S sorbent was packed into 3mL SPE tubes and tannic acid solutions were processed through the tubes.

SPE

SPE Procedure, Using Zymark RapidTrace SPE Workstation

Condition: 2mL MeOH for methanolic samples
2mL MeOH followed by 2mL H₂O for aqueous samples.

Apply Sample: 1mL aliquots of 10mg/mL tannic acid in MeOH or H₂O were applied using a flow rate of 0.75mL/min; fractions were collected after each 1mL of sample was applied.

Breakthrough Results

DPA-6S Sorbent Mass (mg)	Fraction	Methanolic sample Peak Height (mAU)	Aqueous Sample Peak Height (mAU)
300	1	3.7	3.0
	2	8.8	3.5
	3	77.1	6.3
	4	466.5=breakthrough	212
	5	> 3000	262
	6	> 3000	287
	7	> 3000	201.1=breakthrough
600	7	breakthrough	-
	14	-	breakthrough
	2% acid standard	1705	1452

Equivalent results were obtained when using a slower flow rate (0.36mL/min) during sample application, indicating that capacity of DPA-6S for tannic acid does not depend heavily on flow rate. Capacity also seems to be linear with respect to sorbent mass. These results indicate that Discovery DPA-6S products can be used to remove tannic acid and other forms of the compound from aqueous or methanolic solutions, while allowing unretained species to pass through, free of these interfering species.

Tannic Acid from Water or Methanol

HPLC Analysis Conditions

Discovery C 18 column,
15cm x 4.6mm, 5µm particles,
MeOH:H₂O (60:40)
1mL/min, ambient
UV, 254nm
10mL of each fraction

SPE Tube:

DPA-6S
300mg/3mL and 600mg/3mL

Breakthrough Analysis

Breakthrough was defined as the point at which the concentration of tannic acid in the eluent from the tube was greater than that of a 2% solution of tannic acid in methanol or water. Concentrations were determined via HPLC-UV and by comparing peak heights of the tannic acid peak from each fraction to peak heights of tannic acid in the appropriate standard.